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(FILE 'HOME' ENTERED AT 14:21:47 ON 26 JUN 2003)

FILE 'REGISTRY' ENTERED AT 14:22:15 ON 26 JUN 2003

E CYTIDINE MONOPHOSPHATE-2-KETO-3-DEOXY-D-GLYCERO-D-GALACTO-NON

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 14:24:37 ON 26 JUN 2003

SEA (SIALIC ACID OR NEU5AC)

78 FILE ADISCTI
8 FILE ADISINSIGHT
2 FILE ADISNEWS
397 FILE AGRICOLA
182 FILE ANABSTR
265 FILE AQUASCI
158 FILE BIOBUSINESS
11 FILE BIOCOMMERCE
13339 FILE BIOSIS
398 FILE BIOTECHABS
398 FILE BIOTECHDS
3858 FILE BIOTECHNO
1557 FILE CABA
3253 FILE CANCERLIT
18082 FILE CAPLUS
90 FILE CEABA-VTB
22 FILE CEN
19 FILE CIN
250 FILE CONFSCI
1 FILE CROPB
8 FILE CROPU
432 FILE DDFB
317 FILE DDFU
1020 FILE DGENE
432 FILE DRUGB
3 FILE DRUGNL
484 FILE DRUGU
6 FILE DRUGUPDATES
56 FILE EMBAL
10742 FILE EMBASE
2774 FILE ESBIODBASE
107 FILE FEDRIP
78 FILE FROSTI
259 FILE FSTA
449 FILE GENBANK
14 FILE HEALSAFE
509 FILE IFIPAT
1109 FILE JICST-EPLUS
12 FILE KOSMET
2710 FILE LIFESCI
5 FILE MEDICONF
14176 FILE MEDLINE
97 FILE NIOSHTIC
68 FILE NTIS
46 FILE OCEAN
3237 FILE PASCAL
8 FILE PHAR
1 FILE PHARMAML
17 FILE PHIN

132 FILE PROMT
8186 FILE SCISEARCH
2 FILE SYNTHLINE
4011 FILE TOXCENTER
3044 FILE USPATFULL
61 FILE USPAT2
7 FILE VETB
13 FILE VETU
757 FILE WPIDS
757 FILE WPINDEX

L1 QUE (SIALIC ACID OR NEU5AC)

FILE 'CAPLUS, MEDLINE, BIOSIS, EMBASE, SCISEARCH, TOXCENTER, BIOTECHNO,
CANCERLIT, PASCAL' ENTERED AT 14:27:18 ON 26 JUN 2003

L2 456 S L1 AND (NONOIC ACID OR KDN)
L3 45 S L2 AND (CMP-KDN)
L4 11 DUP REM L3 (34 DUPLICATES REMOVED)
L5 8 S L2 AND (SIALIC ACID PHOSPHATE SYNTHASE OR SAS)
L6 2 DUP REM L5 (6

=> d 14 ibib ab 1-11

L4 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 1
ACCESSION NUMBER: 2001:591127 CAPLUS
DOCUMENT NUMBER: 135:300433
TITLE: Molecular cloning of a unique CMP-sialic acid synthetase that effectively utilizes both deaminoneuraminic acid (KDN) and N-acetylneuraminic acid (Neu5Ac) as substrates
AUTHOR(S): Nakata, Daisuke; Munster, Anja-K.; Gerardy-Schahn, Rita; Aoki, Naohito; Matsuda, Tsukasa; Kitajima, Ken
CORPORATE SOURCE: Department of Applied Molecular Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, 464-8601, Japan
SOURCE: Glycobiology (2001), 11(8), 685-692
CODEN: GLYCE3; ISSN: 0959-6658
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB 2-Keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN) is a sialic acid (Sia) that is ubiquitously expressed in vertebrates during normal development and tumorigenesis. Its expression is thought to be regulated by multiple biosynthetic steps catalyzed by several enzymes, including CMP-Sia synthetase. Using crude enzyme preps., it was shown that mammalian CMP-Sia synthetases had very low activity to synthesize CMP-KDN from KDN and CTP, and the corresponding enzyme from rainbow trout testis had high activity to synthesize both CMP-KDN and CMP-N-acetylneuraminic acid (Neu5Ac). To demonstrate if the unique substrate specificity found in the crude trout enzyme is conveyed by a single enzyme, cDNA cloning of trout CMP-Sia synthetase was carried out by PCR-based strategy. The trout enzyme was shown to consist of 432 amino acids with two potential nuclear localization signals, and the cDNA sequence displayed 53.8% identity to that of the murine enzyme. Based on the Vmax/Km values, the recombinant trout enzyme had high activity toward both KDN and Neu5Ac (1.1 vs. 0.68 min⁻¹). In contrast, the recombinant murine enzyme had 15 times lower activity toward KDN than Neu5Ac (0.23 vs. 3.5 min⁻¹). Northern blot anal. suggested that several sizes of the mRNA are expressed in testis, ovary, and liver in a tissue-specific manner. These results indicate that at least one cloned enzyme has the ability to utilize both KDN and Neu5Ac as substrates efficiently and is useful for the prodn. of CMP-KDN.

REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 2
ACCESSION NUMBER: 2001:914804 CAPLUS
DOCUMENT NUMBER: 136:364440
TITLE: Cloning and expression of human sialic acid pathway genes to generate CMP-sialic acids in insect cells
AUTHOR(S): Lawrence, Shawn M.; Huddleston, Kathleen A.; Tomiya, Noboru; Nguyen, Nam; Lee, Yuan C.; Vann, Willie F.; Coleman, Timothy A.; Betenbaugh, Michael J.
CORPORATE SOURCE: Department of Chemical Engineering, The Johns Hopkins University, Baltimore, MD, 21218, USA
SOURCE: Glycoconjugate Journal (2001), 18(3), 205-213
CODEN: GLJOEW; ISSN: 0282-0080
PUBLISHER: Kluwer Academic Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The addn. of sialic acid residues to glycoproteins can affect important protein properties including biol. activity and in vivo circulatory half-life. For sialylation to occur, the donor sugar nucleotide cytidine monophospho-sialic acid (CMP-SA) must be generated and enzymically transferred to an acceptor oligosaccharide. However, examn. of insect cells grown in serum-free medium revealed negligible native levels of the most common sialic acid nucleotide, CMP-N-acetylneuraminic acid (CMP-Neu5Ac). To increase substrate levels, the enzymes of the metabolic pathway for CMP-SA synthesis have been engineered into insect cells using the baculovirus expression system. In this study, a human CMP-sialic acid synthase cDNA was identified and found to encode a protein with 94% identity to the murine homolog. The human CMP-sialic acid synthase (Cmp-Sas) is ubiquitously expressed in human cells from multiple tissues. When expressed in insect cells using the baculovirus vector, the encoded protein is functional and localizes to the nucleus as in mammalian cells. In addn., co-expression of Cmp-Sas with the recently cloned sialic acid phosphate synthase with N-acetylmannosamine feeding yields intracellular CMP-Neu5Ac levels 30 times higher than those obsd. in unsupplemented CHO cells. The absence of any one of these three components abolishes CMP-Neu5Ac prodn. in vivo. However, when N-acetylmannosamine feeding is omitted, the sugar nucleotide form of deaminated Neu5Ac, CMP-2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (CMP-KDN), is produced instead, indicating that alternative sialic acid glycoforms may eventually be possible in insect cells. The human CMP-SAS enzyme is also capable of CMP-N-glycolylneuraminic acid (CMP-Neu5Gc) synthesis when provided with the proper substrate. Engineering the CMP-SA metabolic pathway may be beneficial in various cell lines in which CMP-Neu5Ac prodn. limits sialylation of glycoproteins or other glycans.

REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 3 OF 11 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 3

ACCESSION NUMBER: 2001:374227 CAPLUS

DOCUMENT NUMBER: 135:118967

TITLE: Determination of nucleotides and sugar nucleotides involved in protein glycosylation by high-performance anion-exchange chromatography: Sugar nucleotide contents in cultured insect cells and mammalian cells

AUTHOR(S): Tomiya, Noboru; Ailor, Eric; Lawrence, Shawn M.; Betenbaugh, Michael J.; Lee, Yuan C.

CORPORATE SOURCE: Department of Biology, Johns Hopkins University, Baltimore, MD, 21218, USA

SOURCE: Analytical Biochemistry (2001), 293(1), 129-137
CODEN: ANBCA2; ISSN: 0003-2697

PUBLISHER: Academic Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB We have developed a simple and highly sensitive HPLC method for detn. of cellular levels of sugar nucleotides and related nucleotides in cultured cells. Sepn. of 9 sugar nucleotides (CMP-Neu5Ac, CMP-Neu5Gc, CMP-KDN, UDP-Gal, UDP-Glc, UDP-GalNAc, UDP-GlcNAc, GDP-Fuc, GDP-Man) and 12 nucleotides (AMP, ADP, ATP, CMP, CDP, CTP, GMP, GDP, GTP, UMP, UDP, and UTP) was examd. by reversed-phase HPLC and high-performance anion-exchange chromatog. (HPAEC). Although the reversed-phase HPLC, using an ion-pairing reagent, gave a good sep. of the 12 nucleotides, it did not sep. sufficiently the sugar nucleotides for quantification. On the other hand, the HPAEC method gave an excellent and reproducible sep. of all nucleotides and sugar nucleotides with high sensitivity and reproducibility. We applied the HPAEC method to det. the intracellular sugar nucleotide levels of cultured *Spodoptera frugiperda* (Sf9) and *Trichoplusia ni* (High Five, BTN-TN-5B1-4) insect cells, and

compared them with those in Chinese hamster ovary (CHO-K1) cells. Sf9 and High Five cells showed concns. of UDP-GlcNAc, UDP-Gal, UDP-Glc, GDP-Fuc, and GDP-Man equal to or higher than those in CHO cells. CMP-Neu5Ac was detected in CHO cells, but it was not detected in Sf9 and High Five cells. In conclusion, the newly developed HPAEC method could provide valuable information necessary for generating sialylated complex-type N-glycans in insect or other cells, either native or genetically manipulated. (c) 2001 Academic Press.

REFERENCE COUNT: 71 THERE ARE 71 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1999:558541 CAPLUS

DOCUMENT NUMBER: 132:10152

TITLE: Studies on enzymes involved in the formation and cleavage of the KDN residues in a new class of glycoconjugates containing KDN-glycan chains

AUTHOR(S): Terada, Takaho

CORPORATE SOURCE: Genomic Science Center (GSC), The Institute of Physical and Chemical Research (RIKEN), Saitama, 351-0198, Japan

SOURCE: Trends in Glycoscience and Glycotechnology (1999), 11(59), 147-152

CODEN: TGGLEE; ISSN: 0915-7352

PUBLISHER: FCCA

DOCUMENT TYPE: Journal; General Review

LANGUAGE: English

AB A review with 44 refs. KDN (2-keto-3-deoxy-D-glycero-D-galactononic acid) is a unique form of sialic acid in which the aminoacyl group at C-5 in N-acetylneuraminic acid is substituted by a hydroxyl group. The topics discussed in this review include identification and characterization of CTP: CMP-3-deoxynonulosonate cytidyltransferase (CMP-KDN synthetase) from testis, substrate specificity of CMP-KDN synthetase, identification and characterization of CMP-KDN :lactosylceramide .alpha.2.fwdarw.3 KDN transferase, and studies on the reaction mechanism of a novel sialidase KDNase.

REFERENCE COUNT: 44 THERE ARE 44 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 5 OF 11 PASCAL COPYRIGHT 2003 INIST-CNRS. ALL RIGHTS RESERVED.

ACCESSION NUMBER: 1998-0471689 PASCAL

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TITLE (IN ENGLISH): CHEMOENZYMATIC SYNTHESIS OF SIALYL OLIGOSACCHARIDES AND ANALOGUES INVOLVED IN THE RECOGNITION WITH LECTINS IN METASTATIC PROCESS

TITLE (IN FRENCH): SYNTHESE CHIMIO-ENZYMATIQUE D'OLIGOSACCHARIDES SIALYLES ET D'ANALOGUES IMPLIQUES DANS LA RECONNAISSANCE AVEC LES LECTINES DANS LES PHENOMENES DE METASTASES

AUTHOR: SOMME Valerie; AUGÉ Claudine (dir.)

CORPORATE SOURCE: Universite de Paris 11, Orsay, France (tutelle)

SOURCE: (1998-01), 182 refs. 222 p.

Dissertation Information: Universite de Paris 11.

Orsay. FRA, Th. doct., 98PA112019

DOCUMENT TYPE: Dissertation

BIBLIOGRAPHIC LEVEL: Monographic

COUNTRY: France

LANGUAGE: French

SUMMARY LANGUAGE: French; English

AVAILABILITY: INIST-T 119016, T98PA112019 0000; RBCCN-914712101,

L4 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 4
 ACCESSION NUMBER: 1998:233765 CAPLUS
 DOCUMENT NUMBER: 129:14149
 TITLE: Synthesis of neoglycoconjugates containing deaminated neuraminic acid (KDN) using rat liver .alpha.2,6-sialyltransferase
 AUTHOR(S): Angata, Takashi; Matsuda, Tsukasa; Kitajima, Ken
 CORPORATE SOURCE: Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, Tokyo, 113, Japan
 SOURCE: Glycobiology (1998), 8(3), 277-284
 CODEN: GLYCE3; ISSN: 0959-6658
 PUBLISHER: Oxford University Press
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB 2-Keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN) was introduced into asialotransferrin and N-acetyllactosamine (LacNAc) from **CMP-KDN** by using rat liver Gal.beta.1.fwdarw.4GlcNAc .alpha.2,6-sialyltransferase to form **KDN-transferrin** and **KDN-LacNAc**. These structures contain terminal **KDN** .alpha.2.fwdarw.6Gal-residues, a glycotope that has not yet been described in natural glycoconjugates. **KDN** was transferred to all four Gal residues in asialotransferrin by this enzyme. The incorporation efficiency of **KDN** from **CMP-KDN** into asialotransferrin was about half that of **Neu5Ac** from **CMP-Neu5Ac**, based on the Vmax/Km values for these donor substrates, 0.0527 min⁻¹ and 0.119 min⁻¹, resp. The **KDN.alpha.2.fwdarw.6Gal** linkage was resistant to exosialidase treatment, in contrast to the sensitivity of the **Neu5Ac.alpha.2.fwdarw.6Gal** linkage. Interestingly, Sambucus sieboldiana agglutinin (SSA) was shown to prefer **KDN-transferrin** to the corresponding **Neu5Ac-transferrin**, as estd. by slot-blot anal. The use of an .alpha.2,6-sialyltransferase to synthesize neoglycoproteins contg. **KDN** has not been previously reported. Their facile synthesis using **CMP-KDN** and sialyltransferases with different specificities offers new possibilities to study the function of neo-**KDN**-glycoconjugates, and to explore their use in glycotecnol.

REFERENCE COUNT: 51 THERE ARE 51 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 5
 ACCESSION NUMBER: 1998:533886 CAPLUS
 DOCUMENT NUMBER: 129:276143
 TITLE: Sialyltransferase-catalyzed transfer of **KDN** onto oligosaccharides
 AUTHOR(S): Lubineau, Andre; Somme, Valerie; Auge, Claudine
 CORPORATE SOURCE: URA CNRS 462, Laboratoire de Chimie Organique Multifonctionnelle, Universite PARIS-SUD, Orsay, 91405, Fr.
 SOURCE: Journal of Molecular Catalysis B: Enzymatic (1998), 5(1-4), 235-240
 CODEN: JMCEF8; ISSN: 1381-1177
 PUBLISHER: Elsevier Science B.V.
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 OTHER SOURCE(S): CASREACT 129:276143

AB Sialyltransferases catalyze transfer of N-acetylneuraminic, the most common **sialic acid**, from cytidine 5-monophospho-N-acetylneuraminic acid, onto oligosaccharide chains. 3-Deoxy-.beta.-d-glycero-d-galacto-2-nonulopyranosonic acid (**KDN**), the deaminated analog of N-acetylneuraminic acid, was converted into **CMP-KDN** by a chem. procedure involving **CMP** phosphoramidite.

KDN was then successfully transferred, from **CMP-KDN**, onto Gal.beta.1-3(2OAc)Gal.beta.OCH₃, in porcine liver .alpha.(2-3) sialyltransferase-catalyzed reaction, allowing prepn. of **KDN.alpha.2-3Gal.beta.1-3(2OAc)Gal.beta.OCH₃** in 88% yield. **KDN.alpha.2-6Gal.beta.1-4GlcNAc** could be also prepd. using rat liver sialyltransferase.

REFERENCE COUNT: 13 THERE ARE 13 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 6
 ACCESSION NUMBER: 1996:197572 CAPLUS
 DOCUMENT NUMBER: 124:310943
 TITLE: Substrate specificity of rainbow trout testis **CMP-3-deoxy-D-glycero-D-galacto-nonulosonic acid (CMP-Kdn)** synthetase. Kinetic studies of the reaction of natural and synthetic analogs of nonulosonic acid catalyzed by **CMP-Kdn** synthetase
 AUTHOR(S): Terada, Takaho; Kitajima, Ken; Inoue, Sadako; Koppert, Klaus; Brossmer, Reinhard; Inoue, Yasuo
 CORPORATE SOURCE: Dep. Biophysics and Biochem., Univ. Tokyo, Tokyo, Japan
 SOURCE: European Journal of Biochemistry (1996), 236(3), 852-5
 CODEN: EJBCAI; ISSN: 0014-2956
 PUBLISHER: Springer
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 AB Kinetic data of the activation reaction of several synthetic 3-deoxy-D-glycero-D-galacto-nonulosonic acid (**Kdn**) and N-acetylneuraminic acid (**Neu5Ac**) analogs catalyzed by the rainbow trout testis **CMP-Kdn** synthetase were presented. This enzyme showed broad substrate specificity in terms of substitutions at C4 at C5 position of **Kdn** and **Neu5Ac**. In contrast, calf brain **CMP-N-acetylneuraminic acid** synthetase had narrow substrate specificity, being active only on various N-acyl analogs of **Neu5Ac** and only slightly active on **Kdn** derivs. Usefulness of the trout testis enzyme for synthesis of various **CMP-sialate** analogs, which could be donor substrates for sialyltransferases, was demonstrated.

L4 ANSWER 9 OF 11 MEDLINE
 ACCESSION NUMBER: 95210899 MEDLINE
 DOCUMENT NUMBER: 95210899 PubMed ID: 7696852
 TITLE: Identification, characterization, and developmental expression of a novel alpha 2-->8-**KDN**-transferase which terminates elongation of alpha 2-->8-linked oligo-polysialic acid chain synthesis in trout egg polysialoglycoproteins.
 AUTHOR: Angata T; Kitazume S; Terada T; Kitajima K; Inoue S; Troy F A 2nd; Inoue Y
 CORPORATE SOURCE: Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, Japan.
 CONTRACT NUMBER: AI 09352 (NIAID)
 SOURCE: GLYCOCONJUGATE JOURNAL, (1994 Oct) 11 (5) 493-9.
 Journal code: 8603310. ISSN: 0282-0080.
 PUB. COUNTRY: ENGLAND: United Kingdom
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199505
 ENTRY DATE: Entered STN: 19950510
 Last Updated on STN: 19980206
 Entered Medline: 19950504
 AB A novel glycosyltransferase which catalyses transfer of deaminated neuraminic acid, **KDN** (2-keto-3-deoxy-D-glycero-D-galacto-nononic

acid) from **CMP-KDN** to the non-reducing termini of oligo-polysialyl chains of polysialoglycoprotein (PSGP), was discovered in the ovary of rainbow trout (*Oncorhynchus mykiss*). The **KDN**-transferase activity was optimal at neutral pH, and stimulated 2 to 2.5-fold by 2-5 mM Mg²⁺ or Mn²⁺. Expression of **KDN**-transferase was developmentally regulated in parallel with expression of the alpha 2-->8-polysialyltransferase, which catalyses synthesis of the oligo-polysialyl chains in PSGP. Incorporation of the **KDN** residues into the oligo-polysialyl chains prevented their further elongation, resulting in 'capping' of the oligo-polysialyl chains. This is the first example of a glycosyltransferase that catalyses termination of alpha 2-->8-polysialylation in glycoproteins.

L4 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 7
 ACCESSION NUMBER: 1993:403573 CAPLUS
 DOCUMENT NUMBER: 119:3573
 TITLE: Synthesis of CMP-deaminoneuraminic acid (**CMP-KDN**) using the CTP:TMP-3-deoxynonulosonate cytidyltransferase from rainbow trout testis. Identification and characterization of a **CMP-KDN** synthetase
 AUTHOR(S): Terada, Takaho; Kitazume, Shinobu; Kitajima, Ken; Inoue, Sadako; Ito, Fumio; Troy, Frederic A.; Inoue, Yasuo
 CORPORATE SOURCE: Fac. Sci., Univ. Tokyo, Tokyo, 113, Japan
 SOURCE: Journal of Biological Chemistry (1993), 268(4), 2640-8
 CODEN: JBCHA3; ISSN: 0021-9258
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB The sugar nucleotide, CMP-3-deoxy-D-glycero-D-galacto-2-nonulosonate (**CMP-KDN**) is expected to serve as a donor of **KDN** residues in the synthesis of **KDN**-contg. glycoconjugates. Here, the identification and characterization of CMP-3-deoxynonulosonate cytidyltransferase (**CMP-KDN** synthetase) (I), a novel enzyme responsible for synthesis of **CMP-KDN** from **KDN** and CTP, is reported. I was partially purified from the testis of rainbow trout (*Oncorhynchus mykiss*), where **KDN** gangliosides were 1st discovered, and used to synthesize CMP-[¹⁴C]**KDN**, which was characterized by ¹H NMR. The V_{max}/K_m studies showed that **KDN** was a preferred nonulosonic acid substrate compared to N-acetylneuraminic acid (**Neu5Ac**) or N-glycolylneuraminic acid (**Neu5Gc**) (4.4 .times. 10⁻³ min⁻¹ for **KDN** vs. 2.3 and 1.8 .times. 10⁻³ min⁻¹ for **Neu5Ac** and **Neu5Gc**, resp.). I activity was maximal at pH 9-10 and at 25.degree.. The presence of either Mg²⁺ or Mn²⁺ was essential for I activity. Mg²⁺ (25 mM) stimulated the formation of **CMP-KDN** by >10-fold, yet only stimulated the formation of **CMP-Neu5Ac** and **CMP-Neu5Gc** 4-fold, relative to 1 mM Mg²⁺. A kinetic study using mixed substrates showed that both I and **CMP-Neu5Ac** synthetase activities in the partially purified enzyme were due to the same active site of a single enzyme. In contrast, **Neu5Ac** and **Neu5Gc** were the preferred nonulosonic acid substrates for the calf brain **CMP-sialic acid** synthetase. Thus, mammalian **CMP-sialic acid** synthetases recognize similar, yet distinctively different, substrate specificity determinants. Thus, the trout testis enzyme was considered to synthesize activated sugar nucleotides required for synthesis of both (**KDN**)GM3 and (**Neu5Ac**)GM3. The expression of I was shown to be temporally correlated with development and to parallel the developmental expression of (**KDN**)GM3 in sperm.

L4 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2003 ACS
 ACCESSION NUMBER: 1993:511878 CAPLUS
 DOCUMENT NUMBER: 119:111878
 TITLE: CMP-3-deoxynonulosonate synthetase which catalyzes the

transfer of CMP to KDN from CTP. Partial purification and characterization of the enzyme from rainbow trout testis

AUTHOR(S): Terada, Takaho; Kitazume, Shinobu; Kitajima, Ken; Inoue, Sadako; Ito, Fumio; Troy, Frederic A.; Inoue, Yasuo

CORPORATE SOURCE: Fac. Sci., Univ. Tokyo, Tokyo, 113, Japan

SOURCE: Polysialic Acid (1993), 191-9. Editor(s): Roth, Juergen; Rutishauser, Urs; Troy, Frederick A., II. Birkhaeuser: Basel, Switz.

CODEN: 59FNAM

DOCUMENT TYPE: Conference

LANGUAGE: English

AB The sugar nucleotide, cytidine 5'-(3-deoxy-D-glycero-D-galacto-2-nonulosonic phosphate) (CMP-KDN) is expected to serve as a donor of KDN residues in the synthesis of KDN-contg. glycoconjugates. The identification and characterization of CMP-KDN synthetase, a novel enzyme responsible for synthesis of CMP-KDN from KDN and CTP, is reported. The enzyme was partially purified from the testis of rainbow trout (*Oncorhynchus mykiss*), where KDN-gangliosides were first discovered, and used to synthesize CMP-[¹⁴C]KDN, which was characterized by ¹H NMR. Vmax/Km studies showed that KDN was a preferred nonulosonic acid substrate compared to Neu5Ac or Neu5Gc. In contrast, Neu5Ac and Neu5Gc were the preferred nonulosonic acid substrates for the calf brain CMP-sialic acid synthetase. The presence of either Mg²⁺ or Mn²⁺ is essential for CMP-KDN synthetase activity. Kinetic and substrate specificity studies also showed that the trout testis enzymes could synthesize activated sugar nucleotides required for synthesis of both (KDN)GM3 and (Neu5Ac)GM3. The expression of CMP-KDN synthetase was shown to be temporally correlated with development of sperm.

=> d 16 ibib ab 1-2

L6 ANSWER 1 OF 2 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.DUPLICATE 1
ACCESSION NUMBER: 2003215546 EMBASE
TITLE: Comparative study of carbohydrate chains released from the
oviducal mucins of the two very closely related amphibian
species *Bombina bombina* and *Bombina variegata*.
AUTHOR: Coppin A.; Florea D.; Maes E.; Cogalniceanu D.; Strecker G.
CORPORATE SOURCE: G. Strecker, U. de Glycobiol. Struct. et Fonct., UMR 8576
du CNRS, Univ. des Sci. et Technol. de Lille, 59655
Villeneuve d'Ascq cedex, France. gerard.strecker@univ-
lille1.fr
SOURCE: Biochimie, (2003) 85/1-2 (53-64).
Refs: 21
ISSN: 0300-9084 CODEN: BICMBE
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
010 Obstetrics and Gynecology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The eggs of amphibians are surrounded by an extracellular matrix, termed
jelly coat, which is mainly composed of hydrated mucin-type glycoproteins.
These highly glycosylated molecules are synthesized by the oviduct and
play an important role in the fertilization process. From a structural and
chemical point of view, these oviducal mucins are very different from one
species to another and they could be involved in the species-specificity
of gamete interactions or could influence the parasite tropism. *Bombina*
bombina and *Bombina variegata* are the two most closely related species
within the genus, which hybridize readily in nature. Divergence occurred
during geographic isolation estimated at 2-7 million years ago. The
oviducal mucins of these species have been studied at the carbohydrate
level, and the primary structures of 28 compounds have been established by
NMR spectroscopy. The carbohydrate chains released from the oviducal
mucins of the two species were similar and characterized by the common
sequences GlcNAc(.beta.1-3)[Fuc(.alpha.1-4)]GlcNAc(.beta.1-6) and
GlcNAc(.alpha.1-4)Gal(.beta.1-4)Gal(.beta.1-3) attached to GalNAc-ol (core
2). Nevertheless, some differences confirmed the strict
species-specificity of amphibian oviducal carbohydrate chains observed
previously. On the one hand, the presence of .beta.Gal 1,4-linked to
.beta.GlcNAc in *B. bombina*, but not in *B. variegata*, can indicate that
.beta.4GalT: .beta.GlcNAc and .beta.4GalT: .beta.Gal are two distinct
glycosyltransferases. On the other hand, deaminoneuraminic acid (
Kdn) is present in *B. bombina*, and N-glycolylneuraminic acid
(NeuGc) in *B. variegata*. Although the enzymes involved in the biosynthesis
of **Kdn** are not as well characterized, it can be suggested that
at least one step of the biosynthetic pathway of NeuAc has been disrupted,
leading the *B. bombina* oviducal NeuAc-9-synthase to use Man-6-P as a
substrate, instead of ManNAc-6-P. .COPYRGT. 2003 Editions scientifiques et
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L6 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 2
ACCESSION NUMBER: 2001:914804 CAPLUS
DOCUMENT NUMBER: 136:364440
TITLE: Cloning and expression of human sialic
acid pathway genes to generate CMP-
sialic acids in insect cells
AUTHOR(S): Lawrence, Shawn M.; Huddleston, Kathleen A.; Tomiya,
Noboru; Nguyen, Nam; Lee, Yuan C.; Vann, Willie F.;
Coleman, Timothy A.; Betenbaugh, Michael J.
CORPORATE SOURCE: Department of Chemical Engineering, The Johns Hopkins
University, Baltimore, MD, 21218, USA

SOURCE: Glycoconjugate Journal (2001), 18(3), 205-213
CODEN: GLJOEW; ISSN: 0282-0080
PUBLISHER: Kluwer Academic Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The addn. of **sialic acid** residues to glycoproteins can affect important protein properties including biol. activity and in vivo circulatory half-life. For sialylation to occur, the donor sugar nucleotide cytidine monophospho-**sialic acid** (CMP-SA) must be generated and enzymically transferred to an acceptor oligosaccharide. However, examn. of insect cells grown in serum-free medium revealed negligible native levels of the most common **sialic acid** nucleotide, CMP-N-acetylneuraminic acid (CMP-Neu5Ac). To increase substrate levels, the enzymes of the metabolic pathway for CMP-SA synthesis have been engineered into insect cells using the baculovirus expression system. In this study, a human CMP-**sialic acid** synthase cDNA was identified and found to encode a protein with 94% identity to the murine homolog. The human CMP-**sialic acid** synthase (Cmp-Sas) is ubiquitously expressed in human cells from multiple tissues. When expressed in insect cells using the baculovirus vector, the encoded protein is functional and localizes to the nucleus as in mammalian cells. In addn., co-expression of Cmp-Sas with the recently cloned **sialic acid phosphate synthase** with N-acetylmannosamine feeding yields intracellular CMP-Neu5Ac levels 30 times higher than those obsd. in unsupplemented CHO cells. The absence of any one of these three components abolishes CMP-Neu5Ac prodn. in vivo. However, when N-acetylmannosamine feeding is omitted, the sugar nucleotide form of deaminated Neu5Ac, CMP-2-keto-3-deoxy-D-glycero-D-galactononic acid (CMP-KDN), is produced instead, indicating that alternative **sialic acid** glycoforms may eventually be possible in insect cells. The human CMP-SAS enzyme is also capable of CMP-N-glycolylneuraminic acid (CMP-Neu5Gc) synthesis when provided with the proper substrate. Engineering the CMP-SA metabolic pathway may be beneficial in various cell lines in which CMP-Neu5Ac prodn. limits sialylation of glycoproteins or other glycans.

REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d l4 ibib ab 1-11

L4 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 1

ACCESSION NUMBER: 2001:591127 CAPLUS

DOCUMENT NUMBER: 135:300433

TITLE: Molecular cloning of a unique CMP-**sialic acid** synthetase that effectively utilizes both deaminoneuraminic acid (KDN) and N-acetylneuraminic acid (Neu5Ac) as substrates

AUTHOR(S): Nakata, Daisuke; Munster, Anja-K.; Gerardy-Schahn,

CORPORATE SOURCE: Rita; Aoki, Naohito; Matsuda, Tsukasa; Kitajima, Ken
Department of Applied Molecular Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, 464-8601, Japan

SOURCE: Glycobiology (2001), 11(8), 685-692

CODEN: GLYCE3; ISSN: 0959-6658

PUBLISHER: Oxford University Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB 2-Keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN) is a **sialic acid** (Sia) that is ubiquitously expressed in vertebrates during normal development and tumorigenesis. Its expression

is thought to be regulated by multiple biosynthetic steps catalyzed by several enzymes, including CMP-Sia synthetase. Using crude enzyme preps., it was shown that mammalian CMP-Sia synthetases had very low activity to synthesize **CMP-KDN** from **KDN** and CTP, and the corresponding enzyme from rainbow trout testis had high activity to synthesize both **CMP-KDN** and **CMP-N-acetylneuraminic acid (Neu5Ac)**. To demonstrate if the unique substrate specificity found in the crude trout enzyme is conveyed by a single enzyme, cDNA cloning of trout CMP-Sia synthetase was carried out by PCR-based strategy. The trout enzyme was shown to consist of 432 amino acids with two potential nuclear localization signals, and the cDNA sequence displayed 53.8% identity to that of the murine enzyme. Based on the V_{max}/K_m values, the recombinant trout enzyme had high activity toward both **KDN** and **Neu5Ac** (1.1 vs. 0.68 min⁻¹). In contrast, the recombinant murine enzyme had 15 times lower activity toward **KDN** than **Neu5Ac** (0.23 vs. 3.5 min⁻¹). Northern blot anal. suggested that several sizes of the mRNA are expressed in testis, ovary, and liver in a tissue-specific manner. These results indicate that at least one cloned enzyme has the ability to utilize both **KDN** and **Neu5Ac** as substrates efficiently and is useful for the prodn. of **CMP-KDN**.

REFERENCE COUNT: 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2003 ACS DUPLICATE 2
 ACCESSION NUMBER: 2001:914804 CAPLUS
 DOCUMENT NUMBER: 136:364440
 TITLE: Cloning and expression of human **sialic acid** pathway genes to generate **CMP-sialic acids** in insect cells
 AUTHOR(S): Lawrence, Shawn M.; Huddleston, Kathleen A.; Tomiya, Noboru; Nguyen, Nam; Lee, Yuan C.; Vann, Willie F.; Coleman, Timothy A.; Betenbaugh, Michael J.
 CORPORATE SOURCE: Department of Chemical Engineering, The Johns Hopkins University, Baltimore, MD, 21218, USA
 SOURCE: Glycoconjugate Journal (2001), 18(3), 205-213
 CODEN: GLJOEW; ISSN: 0282-0080
 PUBLISHER: Kluwer Academic Publishers
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 AB The addn. of **sialic acid** residues to glycoproteins can affect important protein properties including biol. activity and in vivo circulatory half-life. For sialylation to occur, the donor sugar nucleotide cytidine monophospho-**sialic acid** (**CMP-SA**) must be generated and enzymically transferred to an acceptor oligosaccharide. However, examn. of insect cells grown in serum-free medium revealed negligible native levels of the most common **sialic acid** nucleotide, **CMP-N-acetylneuraminic acid (CMP-Neu5Ac)**. To increase substrate levels, the enzymes of the metabolic pathway for **CMP-SA** synthesis have been engineered into insect cells using the baculovirus expression system. In this study, a human **CMP-sialic acid** synthase cDNA was identified and found to encode a protein with 94% identity to the murine homolog. The human **CMP-sialic acid** synthase (**Cmp-Sas**) is ubiquitously expressed in human cells from multiple tissues. When expressed in insect cells using the baculovirus vector, the encoded protein is functional and localizes to the nucleus as in mammalian cells. In addn., co-expression of **Cmp-Sas** with the recently cloned **sialic acid** phosphate synthase with N-acetylmannosamine feeding yields intracellular **CMP-Neu5Ac** levels 30 times higher than those obsd. in unsupplemented CHO cells. The absence of any one of these three components abolishes **CMP-Neu5Ac** prodn. in vivo. However, when N-acetylmannosamine feeding is omitted, the sugar nucleotide form of deaminated **Neu5Ac**, **CMP-2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (CMP-KDN)**, is produced

instead, indicating that alternative sialic acid glycoforms may eventually be possible in insect cells. The human CMP-SAS enzyme is also capable of CMP-N-glycolylneuraminic acid (CMP-Neu5Gc) synthesis when provided with the proper substrate. Engineering the CMP-SA metabolic pathway may be beneficial in various cell lines in which CMP-Neu5Ac prodn. limits sialylation of glycoproteins or other glycans.

=> d his

(FILE 'HOME' ENTERED AT 12:53:27 ON 26 JUN 2003)

FILE 'REGISTRY' ENTERED AT 12:53:35 ON 26 JUN 2003
E SIALIC ACID PHOSPHATE SYNTHASE/CN

L1 1 S E4

FILE 'CA, CAPLUS' ENTERED AT 12:54:27 ON 26 JUN 2003

L2 2 S L1 AND (PURIF? OR CHARACT? OR CLON?)

L3 1 DUP REM L2 (1 DUPLICATE REMOVED)

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI,
BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA,
CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB,
DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 12:56:54 ON
26 JUN 2003

SEA (SIALIC ACID PHOSPHATE SYNTHASE)

1 FILE BIOSIS
1 FILE BIOTECHNO
1 FILE CAPLUS
1 FILE EMBASE
1 FILE ESBIODASE
1 FILE MEDLINE
1 FILE SCISEARCH
1 FILE USPATFULL

L4 QUE (SIALIC ACID PHOSPHATE SYNTHASE)

FILE 'BIOSIS, BIOTECHNO, CAPLUS, EMBASE, ESBIODASE, MEDLINE, SCISEARCH,
USPATFULL, ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI,
BIOBUSINESS, BIOCOMMERCE, BIOTECHDS' ENTERED AT 12:59:08 ON 26 JUN 2003

L5 1 S L1

L6 8 S (SIALIC ACID PHOSPHATE SYNTHASE)

L7 2 DUP REM L6 (6 DUPLICATES REMOVED)

=> d 13 ibib ab

L3 ANSWER 1 OF 1 CA COPYRIGHT 2003 ACS DUPLICATE 1
ACCESSION NUMBER: 137:17958 CA
TITLE: Expression of a functional *Drosophila melanogaster*
N-acetylneuraminic acid (Neu5Ac) phosphate synthase
gene: Evidence for endogenous sialic acid biosynthetic
ability in insects
AUTHOR(S): Kim, Kildong; Lawrence, Shawn M.; Park, Jung; Pitts,
Lee; Vann, Willie F.; Betenbaugh, Michael J.; Palter,
Karen B.
CORPORATE SOURCE: Department of Biology, Temple University,
Philadelphia, PA, 19122, USA
SOURCE: Glycobiology (2002), 12(2), 73-83
CODEN: GLYCE3; ISSN: 0959-6658
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In this study, we report the first **cloning** and
characterization of a N-acetylneuraminic acid phosphate synthase
gene from *Drosophila melanogaster*, an insect in the protostome lineage.
The gene is ubiquitously expressed at all stages of *Drosophila* development
and in Schneider cells. Similar to the human homolog, the gene encodes an
enzyme with dual substrate specificity that can use either
N-acetylmannosamine 6-phosphate or mannose 6-phosphate to generate
phosphorylated forms of both the sialic acids, N-acetylneuraminic acid and
2-keto-3-deoxy-D-glycero-D-galacto-nononic acid, resp., when expressed in
either bacterial or baculoviral expression systems. The identification of
a functional sialic acid synthase in *Drosophila* indicates that insects
have the biosynthetic capability to produce sialic acids endogenously.
Although sialylation is widely distributed in organisms of the deuterostome
lineage, genetic evidence concerning the presence or absence of sialic
acid metab. in organisms of the protostome lineage has been lacking.
Homol. searches of the *Drosophila* genome identified putative orthologues
of other genes required for sialylation of glycoconjugates.
REFERENCE COUNT: 66 THERE ARE 66 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d 17 ibib ab 1-2

L7 ANSWER 1 OF 2 USPATFULL

ACCESSION NUMBER: 2002:258816 USPATFULL
TITLE: Engineering intracellular sialylation pathways
INVENTOR(S): Betenbaugh, Michael J., Baltimore, MD, UNITED STATES
Lawrence, Shawn, Dobbs Ferry, NY, UNITED STATES
Lee, Yuan C., Timonium, MD, UNITED STATES
Coleman, Timothy A., Gaithersburg, MD, UNITED STATES

| | NUMBER | KIND | DATE |
|---------------------|----------------|------|--------------|
| PATENT INFORMATION: | US 2002142386 | A1 | 20021003 |
| APPLICATION INFO.: | US 2001-930440 | A1 | 20010816 (9) |

| | NUMBER | DATE |
|-----------------------|-----------------|---------------|
| PRIORITY INFORMATION: | US 2000-227579P | 20000825 (60) |
| | US 1999-169624P | 19991208 (60) |
| | US 1999-122582P | 19990302 (60) |

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: HUMAN GENOME SCIENCES INC, 9410 KEY WEST AVENUE,
ROCKVILLE, MD, 20850
NUMBER OF CLAIMS: 47
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 40 Drawing Page(s)
LINE COUNT: 4472

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods for manipulating carbohydrate processing pathways in cells of interest are provided. Methods are directed at manipulating multiple pathways involved with the sialylation reaction by using recombinant DNA technology and substrate feeding approaches to enable the production of sialylated glycoproteins in cells of interest. These carbohydrate engineering efforts encompass the implementation of new carbohydrate bioassays, the examination of a selection of insect cell lines and the use of bioinformatics to identify gene sequences for critical processing enzymes. The compositions comprise cells of interest producing sialylated glycoproteins. The methods and compositions are useful for heterologous expression of glycoproteins.

L7 ANSWER 2 OF 2 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1

ACCESSION NUMBER: 2002:56015 BIOSIS
DOCUMENT NUMBER: PREV200200056015
TITLE: Cloning and expression of human sialic acid pathway genes to generate CMP-sialic acids in insect cells.
AUTHOR(S): Lawrence, Shawn M.; Huddleston, Kathleen A.; Tomiya, Noboru; Nguyen, Nam; Lee, Yuan C.; Vann, Willie F.; Coleman, Timothy A.; Betenbaugh, Michael J. (1)
CORPORATE SOURCE: (1) Department of Chemical Engineering, Johns Hopkins University, 3400 N. Charles St., Baltimore, MD, 21218: beten@jhu.edu USA
SOURCE: Glycoconjugate Journal, (March, 2001) Vol. 18, No. 3, pp. 205-213. print.
ISSN: 0282-0080.

DOCUMENT TYPE: Article
LANGUAGE: English

AB The addition of sialic acid residues to glycoproteins can affect important protein properties including biological activity and in vivo circulatory half-life. For sialylation to occur, the donor sugar nucleotide cytidine monophospho-sialic acid (CMP-SA) must be generated and enzymatically transferred to an acceptor oligosaccharide. However, examination of insect cells grown in serum-free medium revealed negligible native levels of the

most common sialic acid nucleotide, CMP-N-acetylneuraminic acid (CMP-Neu5Ac). To increase substrate levels, the enzymes of the metabolic pathway for CMP-SA synthesis have been engineered into insect cells using the baculovirus expression system. In this study, a human CMP-sialic acid synthase cDNA was identified and found to encode a protein with 94% identity to the murine homologue. The human CMP-sialic acid synthase (Cmp-Sas) is ubiquitously expressed in human cells from multiple tissues. When expressed in insect cells using the baculovirus vector, the encoded protein is functional and localizes to the nucleus as in mammalian cells. In addition, co-expression of Cmp-Sas with the recently cloned

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LANGUAGE: English
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